EXHIBIT A

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The 2-Kilobase Intron of the Herpes Simplex Virus Type 1 Latency-Associated Transcript Has a Half-Life of Approximately 24 Hours in SY5Y and COS-1 Cells

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The herpes simplex virus type 1 (HSV-1) 2-kb latency-associated transcript (LAT) is a stable intron, which accumulates in cells both lytically and latently infected with HSV-1. We have used a tetracycline-repressible expression system to determine the half-life of the 2-kb LAT RNA intron in the human neuroblastoma cell line SY5Y. Using Northern hybridization analyses of RNA isolated from transiently transfected SY5Y cells over time after repression of LAT expression, we measured the half-life of the 2-kb LAT to be approximately 24 h. Thus, unlike typical introns that are rapidly degraded in a matter of seconds following excision, the 2-kb LAT intron has a half-life similar to those of some of the more stable cellular mRNAs. Furthermore, a similar half-life was measured for the 2-kb LAT in transiently transfected nonneuronal monkey COS-1 cells, suggesting that the stability of the 2-kb LAT is neither cell type nor species specific. Previously, we found that the determinant responsible for the unusual stability of the 2-kb LAT maps to the 3′ terminus of the intron. At this site is a nonconsensus intron branch point located adjacent to a predicted stem-loop structure that is hypothesized to prevent debranching by cellular enzymes. Here we show that mutations which alter the predicted stem-loop structure, such that branching is redirected, either reduce or abolish the stability of the 2-kb LAT intron.

Individuals infected with herpes simplex virus type 1 (HSV-1) harbor a lifelong latent infection of neurons in the sensory ganglia, with occasional recurrences of an acute infection at the site of initial infection (for a review, see reference 47). In a latent infection of neurons, the HSV-1 latency-associated transcripts (LATs) are the only transcripts produced at readily detectable levels (12, 36, 39). Two major species of LAT are observed during a latent infection and have apparent molecular sizes of 2.0 and 1.5 kb (29, 36–39, 45). Expression of LAT, however, is not exclusive to latent infections, since the 2-kb LAT species is also expressed during acute infections with late-gene kinetics (37, 44). These RNAs are now known to be introns (14, 50), with the less abundant 1.5-kb LAT being encoded entirely within the 2-kb LAT (50). The high level of these LAT species within the nuclei of latently infected cells suggests that these introns are expressed at very high levels or are unusually stable. In fact, it has been shown that these introns are indeed unusually stable (21, 30, 48, 50). This is in contrast to typical cellular introns that are rapidly degraded following excision from the pre-mRNA (23).

The function of LAT during latent and acute infections is not known, although recent studies have shed light upon why the LAT intron is stable. Two independent studies have mapped the branch point of the 2-kb LAT intron to either a guanosine (50) or an adenosine (48) residue present within a nonconsensus branch point sequence. This nonconsensus branch point sequence is located immediately upstream of a potential stem-loop structure with a predicted ΔG of −39.7 kcal/mol (50). Although the precise branch point nucleotide is disputed, it is clear that the unusual branch point region and/or sequences within the stem-loop are required for stability of the intron (21). The mechanism of the stability is hypothesized to involve the inability of the intron lariat to be debranched in vivo (30, 49). In this regard, guanosine branch points are poor substrates for mammalian debranching activity and are debranched at approximately 50% of the rate for adenosine branch points in vitro (4). Furthermore, the stable stem-loop structure that potentially forms between the LAT intron branch point and its polypyrimidine tract may potentially mediate the unusual stability of this intron by further blocking the progression of debranching enzymes (21, 50).

Although the stability of the LATs has been confirmed (21, 50), a half-life value has not been measured. Here, we have employed a tetracycline-repressible system to measure the half-life of the 2-kb LAT intron in the human neuroblastoma cell line SY5Y. In contrast to most cellular introns (23), the HSV-1 2-kb LAT is extraordinarily stable, with a measured half-life of approximately 24 h, which is similar to the half-lives of the more stable cellular mRNAs. This stability is not cell type or species specific, because a half-life of approximately 24 h was measured in transiently transfected monkey COS-1 cells. However, the selected branch point does correlate with the stability of the intron. Specifically, a guanosine branch

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